Microbial Ecology of Arsenic Contaminated Aquifers and Potential Bioremediation

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Summary

Microbial iron and arsenic cycling plays an important role in the (im)mobilization of arsenic in aquifers. Consequently, the fate of arsenic present in groundwater can be influenced by various oxidizing and reducing microbial activities along with hydrogeochemistry. Arsenic contamination in Southeast and South Asia is itself thought to be biogeogenic. In Bangladesh, aquifer geology is heterogeneous leading to various niches at different locations. We would expect that this heterogeneity also leads to differences between the microbial communities residing in the various niches. As a result the arsenic concentration would then vary along with local geochemistry and this affects the aquifers used as sources for drinking water. Understanding of the microbial ecology of the arsenic cycle of oxidation, reduction, influx and precipitation in relation to the contamination of drinking water in Bangladesh on the one hand, and the potential contribution of arsenic cycling microorganisms to arsenic remediation on the other hand, is still limited. Important is the integral understanding of all the contributing processes, i.e. the microbial systems ecology. In view of their interference with the arsenic cycle, both the microbe-mediated iron and sulfur cycles add yet another level of complexity to this system.

The objectives of the research described in this thesis were to enhance the insights in the microbial ecology of arsenic-contaminated aquifers used for drinking water in Bangladesh and to understand the microbial potential for arsenic bioremediation and bioaugmentation thereof. For several arsenic-contaminated geographical regions of Bangladesh we studied the hydrochemistry, the existing microbial community structure, and the potential of that community to amplify its functional organism-groups capable of arsenic and iron cycling. We also inspected an existing iron-cycling based abiotic arsenic decontamination technology. Both field-scale hydrochemical measurements and laboratory experiments were performed. We assayed the presence and composition of arsenic- and iron-oxidizing and -reducing microbial communities in terms of culture-independent 16S rRNA-based genes, and the group-specific functional genes encoding arsenite oxidase (*aioA*) and arsenate reductase (*arrA*). We did the same for cultures subjected to attempts to enrich the original groundwater samples for arsenic or iron metabolizing microorganisms. In view of the diversity of the geochemical niches for the organisms, the cultivation conditions ranged from aerobic, microaerobic, to strictly anaerobic, from chemolithoautotrophic to heterotrophic, and from arsenite oxidizing to arsenate reducing conditions along with ferrous iron to ferric iron. We also investigated the impact of long term running of a subsurface arsenic removal (SAR) treatment unit, on the local hydrochemistry and microbiology and vice versa, i.e. the influence of microbes on arsenic removal efficiency.

Chapter 1 comprises a general introduction to this thesis, addressing relevant subjects such as the history of water usages in Bangladesh, the occurrence and distribution of arsenic in drinking water systems, the negative effects of arsenic on health, arsenic chemistry, and groundwater hydrochemistry in relation to arsenic mobility and dynamics. The Introduction Chapter also describes the cost-effective *in situ* subsurface arsenic removal (SAR) technology recently introduced to Bangladesh, as well as microbiological processes that could potentially benefit arsenic bioremediation through oxidation of ferrous iron and

arsenite and other microbiological processes that could potentially contribute negatively by mobilizing arsenic via reduction of ferric iron and arsenate.

Chapter 2 makes an inventory of the arsenic- and iron-cycling microbial communities in arsenic-contaminated aquifers used for drinking water in Bangladesh. Through a cultivation-independent survey covering 24 drinking water wells in several geographical regions of Bangladesh, it obtains a wealth of information on the microbial community structure in these aquifers, and on the specific functional organism-groups capable of the oxidation or reduction of arsenic or iron. Groups of microorganisms, identified either by group-specific 16S rRNA or by the amplification if the genes encoding arsenite oxidase (*aioA*) and respiratory arsenate reductase (*arrA*), occurred in at least 79 % of the investigated samples. Putative arsenate reducers and iron-oxidizing *Gallionellaceae* were present at low diversity, while more variation was revealed within and between samples in potentially arsenite-oxidizing microorganisms and iron-reducing *Geobacteraceae*. Contrary to the above expectations, relations between community composition on the one hand and hydrochemistry on the other hand were not evident, apart from an impact of salinity on iron-cycling microorganisms. Therefore, in order to understand the role of arsenic and iron cycling in arsenic-contaminated groundwater, it appeared essential to follow-up our previous cultivation-independent work on arsenic and iron cycling with cultivation studies. Accordingly, the aims were to survey the abundance, distribution and diversity of cultivatable arsenic- and iron-oxidizing and reducing microorganisms in arsenic-contaminated drinking water wells in Bangladesh.

The activities of iron-oxidizing and iron-reducing microorganisms may also impact the fate of arsenic in groundwater. In **Chapter 3**, we complemented a previous cultivation-independent microbial community survey covering 22 arsenic-contaminated drinking water wells in Bangladesh, with the characterization of cultures favoring microaerophilic iron oxidizers and anaerobic iron reducers, conducted on the same water samples as used in **Chapter 2** and **Chapter** 4. All enrichments investigated, revealed a potential for microbial iron oxidation and reduction. Microbial communities were phylogenetically diverse within and between enrichments as was also observed in the previous cultivation-independent analysis of the water samples from which these cultivations were derived. Enrichments uncovered a larger diversity in iron-cycling microorganisms than previously indicated. Iron-oxidizing oxygen-gradient tube enrichments were dominated by *Comamonadaceae* and *Rhodocyclaceae* instead of *Gallionellaceae*. The iron-reducing enrichments contained several 16S rRNA gene sequences, which related most closely to *Acetobacterium*, *Clostridium*, *Bacillus*, *Rhizobiales*, *Desulfovibrio*, *Bacteroides* and *Spirochaetes*, as well as to well-known dissimilatory iron-reducing *Geobacter* and *Geothrix* species. Forty-five percent of the ferrous iron-oxidizing enrichments were home to the gene encoding arsenite oxidase.

Phylogenetic properties provide insufficient information for inferring the potential of the microbial population of aquifers for arsenic oxidation or reduction. Therefore in **Chapter 4**, we further complemented the cultivation-independent microbial community survey covering 22 arsenic-contaminated drinking water wells in Bangladesh of Chapter 2 with new information relating more directly to activity. This involved the characterization of cultivation favoring aerobic or denitrifying chemolithoautotrophic arsenite oxidizers, or

aerobic and anaerobic heterotrophic arsenite oxidizers, or anaerobic arsenate reducers. Nearly all investigated enrichments revealed a potential for microbial arsenite oxidation and/or arsenate reduction. Enrichments were analysed further by 16S rRNA and functional gene amplification. The microbial communities in the aquifers were phylogenetically diverse within and between enrichments. Cultivations revealed a larger diversity of arsenic-cycling microorganisms compared to their original water samples. The enrichments also recovered other and more AioA phylotypes than had been detected in our previous molecular survey (**Chapter 2**) of groundwater samples. Most cultivation conditions disclosed both an alpha (within well) and a gamma (between wells) diversity in arsenite oxidizing AioA phylotypes between the different types of enrichment, such that also the beta diversity (i.e. the gamma diversity relative to the alpha ratio) was substantial. Anaerobic-arsenate reducing enrichment revealed little 16S rRNA and *arrA* gene diversity in each well, although the beta diversity remained substantial: the wells differed greatly between each other.

Removal of subsurface arsenic by injection with oxygenated groundwater has been proposed as a viable cost-effective *in situ* technology, named "SAR" (subsurface arsenic removal) for obtaining "safe" drinking water in Bangladesh. While the oxidation of aqueous ferrous iron to solid ferric iron (oxyhydr)oxides, which tend to precipitate and provide sorption sites to arsenic, has been presented as if driven exclusively by abiotic reactions, metal-cycling microorganisms might affect the arsenic removal. In order to investigate the potential microbial role in SAR, we conducted a field experiment along with cultivation-independent and cultivation-dependent molecular analyses. We report on this in **Chapter 5**, where we also discuss our bioreduction experiment with water and sediment sampled from either the SAR or a reference well. Our molecular analyses suggested that robust microbial changes were very limited in SAR. Our data also suggested that running of a SAR unit on small scale could not stimulate growth of the metal oxidizing microorganisms sufficiently for them to provide arsenic-poor, as well as microbiologically safe drinking water, at least not on the safe side of the WHO guidelines. Moreover, we did not find any significant increase with time in any microbial contribution to arsenic removal efficiency in SAR. The bioremobilization experiment suggests that microbes-mediated iron and arsenic reduction may constitute a potential liability when the SAR is stopped.

Chapter 6 is a general discussion about the main findings of this thesis and on how these findings contribute to the understanding of rational bioremediation of arsenic *in situ* and *ex situ*. A large number of *ex situ* treatment technologies have been developed to provide arsenic free safe drinking water to the population. But most of the technologies are not sustainable and affordable to the generally poor and rural population of Bangladesh, due to high maintenance costs and required logistic care. The arsenite and ferrous iron oxidizing bacteria that we identified should be of interest to emerging technologies in arsenic bioremediation at the community level. Using ammonia oxidizing bacteria to produce nitrate biologically might constitute an alternative and cost-effective strategy towards anaerobic microbe-mediated arsenite and ferrous iron oxidation. We discuss how the SAR technology might be amended so as to incorporate this and so as to reduce the risk of arsenite emergence after stopping of the SAR.